

Egg Longevity and Time-Integrated Fertilization in a Temperate Sea Urchin (*Strongylocentrotus droebachiensis*)

SUSANNE K. MEIDEL* AND PHILIP O. YUND

School of Marine Sciences, Darling Marine Center, University of Maine, Walpole, Maine 04573

Abstract. Recent field experiments have suggested that fertilization levels in sea urchins (and other broadcast spawners that release their gametes into the water column) may often be far below 100%. However, past experiments have not considered the potentially positive combined effects of an extended period of egg longevity and the release of gametes in viscous fluids (which reduces dilution rates). In a laboratory experiment, we found that eggs of the sea urchin *Strongylocentrotus droebachiensis* had high viability for 2 to 3 d. Fertilization levels of eggs held in sperm-permeable egg baskets in the field and exposed to sperm slowly diffusing off a spawning male increased significantly with exposure from 15 min to 3 h. In a field survey of time-integrated fertilizations (over 24, 48, and 72 h) during natural sperm release events, eggs held in baskets accrued fertilizations over as much as 48 h and attained fairly high fertilization levels. Our results suggest that an extended period of egg longevity and the release of gametes in viscous fluids may result in higher natural fertilization levels than currently expected from short-term field experiments.

Introduction

Recent work has started to explore the fertilization dynamics of free-spawning marine organisms that release one or both gametes into the water column (e.g., algae: Pearson and Brawley, 1996; corals: Lasker *et al.*, 1996; starfish: Babcock *et al.*, 1994; sea urchins: Levitan *et al.*, 1992; ascidians: Yund, 1998; fish: Petersen *et al.*, 1992). Although the details of scientific approaches vary, studies can be

broadly grouped into experiments in which a limited number of manipulated organisms are induced to spawn, and surveys of natural spawning events (Levitian, 1995; Yund, 2000). Experimental studies that control spawning synchrony and spatial relationships to test specific mechanistic hypotheses generally suggest that fertilization levels may be limited by sperm availability unless males and females spawn simultaneously, at close range, or under nearly ideal flow conditions (see Levitan and Petersen, 1995; and Yund, 2000, for reviews). In contrast, many surveys of natural spawns report fairly high fertilization levels, at least at the times and places in which most members of a population spawn (Yund, 2000). However, comparisons between existing experiments and surveys are complicated by two major factors. First, results from experimental studies can successfully predict fertilization levels in natural spawns only if experimental conditions (both biotic and abiotic) accurately mimic natural spawning conditions; however, experiments often circumvent reproductive strategies that may have evolved to enhance fertilization (Yund, 2000). Second, experiments and surveys are rarely conducted with the same species, so it is virtually impossible to distinguish between taxonomic and methodological effects in existing studies.

Echinoderms have proven to be a particularly valuable model system for short-term field experiments, and experimental fertilization data from echinoderms generally support the paradigm of severe sperm limitation under a wide range of flow and population conditions (e.g., Pennington, 1985; Levitan, 1991; Levitan *et al.*, 1992; Wahle and Peckham, 1999; but see Babcock *et al.*, 1994). However, there are no published surveys of fertilization levels in natural spawns of echinoderms. The absence of survey data is probably due in part to a lack of information on temporal spawning patterns and the proximate environmental cues that initiate spawning (though multiple cues have been

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* To whom correspondence should be addressed. E-mail: meidel@maine.edu

proposed and investigated: Himmelman, 1975; Starr *et al.*, 1990, 1992, 1993).

Two interrelated adaptations that have been largely bypassed in previous experimental studies may have considerable effects on fertilization levels in natural spawns of temperate echinoderms. The first is an extended period of egg viability, which potentially allows fertilizations to accrue over time. Short-term experiments make one or both of the following assumptions: that most eggs are fertilized within the first few seconds of release (Denny and Shibata, 1989; Levitan *et al.*, 1991) and that gametes are quickly diluted to concentrations below which fertilization can occur. Consequently, extended egg viability has implicitly been presumed to have little influence on fertilization levels in the field. Meanwhile, recent estimates of egg longevity have steadily extended what was presumed to be a relatively short period of viability. Pennington (1985) reported a minimum viability period of 24 h for eggs of the temperate sea urchin *Strongylocentrotus droebachiensis* (Müller), and eggs of a West coast sea urchin are now known to be viable for up to 2 wk when stored under axenic conditions (Epel *et al.*, 1998). If eggs can be fertilized for a long period of time, extended or repeated exposure of eggs to sperm during long-duration spawning events (or events in which multiple males spawn successively) could result in high time-integrated levels of fertilization, even if sperm are limiting in the short term.

A second adaptation that may interact with extended egg longevity to increase fertilization levels is the release of gametes in viscous fluids, which reduces gamete dilution rates and potentially increases the duration of egg exposure to sperm. Thomas (1994) has shown that three species of sea urchins (*Tripneustes gratilla*, *Echinometra mathaei*, and *Colobocentrotus atratus*) release gametes in such viscous fluids that eggs and sperm remain on the test and spines at current speeds less than $0.13 \text{ m} \cdot \text{s}^{-1}$. When the current speed increases, gametes are transported away from this reservoir in long (3–4 cm) strings or clumps, which led Thomas (1994) to hypothesize that sea urchins may achieve high fertilization levels if gametes encounter each other in these structures. Sperm concentrated in clumps presumably also have greater longevity because of a reduction in the respiratory dilution effect (Chia and Bickell, 1983). In contrast to natural sperm release, fertilization experiments often mimic "males" with syringes from which diluted gametes are extruded at a fixed (and fast) rate, thus circumventing the potentially beneficial effect of "sticky" sperm that cling to the test and spines and slowly diffuse away.

In this study, we investigate the effects of these two aspects of sea urchin reproductive biology on fertilization levels in *Strongylocentrotus droebachiensis*. We initially determine the duration of egg viability at two points during the reproductive season. We then explore whether extended (3 h) exposure of eggs to sperm diffusing off a male sea

urchin enhances fertilization levels relative to short-term (15 min) contact at various downstream distances. Finally, we use the full period of egg viability to assay time-integrated fertilization levels during natural sperm release events in small populations and use the distribution of developmental stages in these field samples to evaluate the temporal distribution of fertilization events.

Materials and Methods

General procedures

To obtain fresh eggs and sperm for use in experiments and field sampling, sea urchins (*Strongylocentrotus droebachiensis*) were injected through the peristomial membrane with 0.2–2.0 ml of 0.5 M KCl. Females spawned into 50-ml glass beakers containing chilled seawater that had been aged (~15–20 h; hereafter referred to as aged seawater) to eliminate ambient sperm. Female spawn was checked to confirm the absence of immature oocytes (as indicated by the presence of a large nucleus and nucleolus) and then washed three times with aged seawater. Dry sperm was pipetted directly from the aboral surface of spawning males and kept refrigerated until use (maximum 2 h).

To assay fertilization levels in the field, unfertilized eggs were deployed in sperm-permeable containers. These egg baskets consisted of a 0.1-m-long frame of PVC pipe (internal diameter 0.05 m) with the sides (~90% of circumference) cut away, covered with 35-μm Nitex mesh (after Wahle and Peckham, 1999, as modified from Levitan *et al.*, 1992), and two Styrofoam floats attached for positive buoyancy. Baskets were suspended from the surface or deployed on the bottom in different spatial arrangements as described in the following sections.

Egg longevity

To determine the viability period of eggs of *Strongylocentrotus droebachiensis*, we performed laboratory experiments at the beginning (experiment 1: February 28 to March 2, 2000) and in the middle (experiment 2: March 28 to April 1, 2000) of the spawning season along the coast of Maine (March to May, Cocanour and Allen, 1967). In each experiment, 120 μl of freshly spawned eggs (mean ± SE of 1651 ± 69 eggs) from each of four females were added to 10 ml aged seawater (aerated for 1 h prior to use) in 20-ml glass scintillation vials. At the start of each experiment (0 h) and after 24, 48, 72, and 96 h (experiment 2 only), eggs in each of four replicate vials per female (only one replicate per female at 0 h in experiment 1) were fertilized with 20 μl of a 10-fold sperm dilution (10 μl fresh dry sperm from 3 males, 90 μl aged seawater). Vials were gently agitated three times during a 15-min period, following which the fertilization process was stopped with the addition of 2.5 ml 37% formaldehyde. At each time point, one additional vial

per female was fixed without fertilization, as a control for false fertilization envelopes (from causes such as egg damage or low egg quality). Vials were kept at ambient seawater temperature (1° – 3°C) during both experiments. Fertilization levels were calculated as the percentage of a random subsample of 300 eggs with a fertilization envelope.

Two-way analyses of variance (ANOVA) with the fixed factors Female (four levels) and Time (three levels in experiment 1; five in experiment 2) were used to analyze variation in fertilization levels (% fertilization). To achieve homogeneity of variances, percent fertilization values were arcsine transformed for experiment 1 (O'Brien's test, $F = 1.20$, $P > 0.32$) but not transformed for experiment 2 (O'Brien's test, $F = 1.35$, $P > 0.19$). The Student-Newman-Keuls (SNK) test was used for *post-hoc* comparisons of levels within main effects in the absence of a significant interaction effect.

Cumulative fertilization in the field: 15 min vs 3 h

In this experiment, we determined whether extended (3 h) exposure of eggs in baskets to sperm from a spawning male enhanced fertilization levels relative to short-term (15 min) exposure. We constructed a fertilization platform that was mounted on a concrete block ($L \times W \times H$: 0.36 m \times 0.33 m \times 0.14 m) deployed by a rope. The platform consisted of a pine board (1.59 m \times 0.24 m \times 0.02 m) bolted to the concrete block so that it extended 0.31 m upstream of the block and 0.92 m downstream. The board housed one male and two female stations. The male station was simply a surface-mounted PVC plate (0.08 m \times 0.12 m \times 0.003 m), located 0.30 cm from the upstream end of the board, to which a spawning male could be fastened. Female stations consisted of eyebolts anchoring ropes that extended to the surface and were located 0.3 and 1.0 m downstream of the male station.

Experiments were performed on a sandy substratum below the dock of the University of Maine's Darling Marine Center in the Damariscotta River estuary (ME, $43^{\circ}50'N$, $69^{\circ}33'W$) at a depth of 4.30 m at mean low water (MLW). For each trial ($n = 8$), four egg baskets (two side by side ~ 0.05 m above the platform at each of two female stations) containing 500 μl freshly spawned eggs (mean \pm SE: 7613 ± 455 eggs) from one female were attached to the eyebolts. A male was induced to spawn by injection of 2.5–4.5 ml 0.5 M KCl and then attached to the male station with rubber bands. The fertilization platform was then immediately deployed. In addition to the platform, two mobile female stations (baskets on weighted lines with the lower basket 0.35 m above the substratum) were deployed 2 m upstream (control for ambient sperm: one basket) and ~ 2.60 m downstream (two baskets spaced 0.1 m apart vertically, omitted from trial 1) from the male station. After 15 min, one egg basket from each of the three downstream

female stations was retrieved without disturbing the remainder of the array, by pulling it to the surface on its own line. The remaining baskets were retrieved after 3 h, and the presence or absence of sperm on the aboral surface of the male was recorded. Eggs were immediately collected and fixed with formaldehyde. To determine fertilization levels, 300 eggs per vial (200–300 in five cases, 154 in one case) were randomly sampled and scored for the presence or absence of a fertilization envelope. Where sufficient numbers of eggs were retrieved (82% of baskets), small subsamples were taken before fixation and scored after about 15–20 h for the presence or absence of later developmental stages.

During trials 2 through 8, current velocity was recorded with a 3D-ACM acoustic-doppler current meter (Falmouth Scientific). Each trial took place around mid-tide (*i.e.*, commenced ~ 1.5 h after high [or low] water and ended ~ 1.5 h before low [or high] water) to minimize variation in the flow regime.

Three laboratory controls (held at $\sim 3^{\circ}\text{C}$), consisting of 200 μl freshly spawned eggs in 10 ml aged seawater, were assayed for (1) fertilization at the start of each trial; (2) fertilization at the end of each trial; and (3) the presence of false fertilization envelopes, scored twice (after retrieval of 15 min and 3 h samples). Laboratory controls were scored in the same manner as field samples.

A two-way ANOVA with the fixed factors Time (two levels) and Distance (three levels) was used to determine differences in fertilization levels (%) in field samples. Percent fertilization values were arcsine transformed prior to analysis to achieve homogeneity of variances (O'Brien's test, $F = 0.94$, $P > 0.47$).

Sperm availability in nature

We measured cumulative (over 24, 48, or 72 h) fertilization levels of eggs retained in baskets during natural spawning events of *Strongylocentrotus droebachiensis*. This sampling design is a hybrid between an experiment and a true survey of natural spawns, because any sperm present were naturally released, but egg locations were under experimental control. Sampling started in mid-February and ended in early April in 1999 and 2000 but varied in intensity (both spatial and temporal) during the two years. In 1999, samples were collected at a single station at Christmas Cove (ChC, mouth of the Damariscotta River estuary); in 2000, samples were collected from three stations at ChC and four stations at Clarks Cove (CIC, 1 km seaward of the Darling Marine Center and ~ 9 km from the ChC site). Both sites were relatively sheltered with a sandy substratum, and surveys of the immediate surroundings indicated the absence of sea urchin populations other than those sampled (pers. obs.). A small population of *S. droebachiensis* (~ 150 animals in 1999, ~ 60 in 2000) occurred naturally at ChC. At CIC, we

released about 350 sea urchins on a rock ledge around the lower low water line on January 29, 2000, but this population appeared to have declined to about 30 animals by April 7, 2000.

At each site, multiple stations were positioned to provide samples at different nominal distances from the sea urchins. At ChC, station 1 was within 1 m of a rock wall that was inhabited by sea urchins during the autumn months; station 2 was on the shoreward end of a floating dock, 5 m straight offshore of the wall; and station 3 was on the seaward end of the same dock, about 13 m from the wall. The shallow depth of station 1 (1.4 m at MLW) allowed sampling at only one depth (0.15 to 0.35 m above the substratum). At stations 2 and 3, we sampled the surface waters during each interval (1.4 to 6.2 m above the substratum, depending on the tidally variable water depth); at times of anticipated sperm presence (based on 1999 results) we also sampled the bottom water 0.15 to 0.35 m above the substratum. During 1999, only station 3 was sampled, and egg baskets were deployed only near the surface. Because the sea urchins were free to move, the positions of our stations relative to spawning males could not be known precisely. However, likely locations can be inferred from sea urchin movement patterns. In 1999, sea urchins mainly remained on the rock wall or wandered between stations 1 and 2, whereas in 2000 many animals spent the spawning season on a piling adjacent to station 2.

We employed a similar sampling scheme at CIC, with minor modifications to accommodate local dock structures. Station 1 was within 1 m of the rock ledge to which sea urchins were transplanted; station 2 was 1 m straight offshore of station 1 (along a fixed wooden dock); and stations 3 and 4 were on floating docks about 12 m from station 1, at 45° angles to either side of the transect from stations 1 to 2. Because of minimal water depth (1.0 to 1.4 m at MLW), all stations were sampled at only a single depth (stations 1 and 2: 0.15 to 0.35 m above the substratum; stations 3 and 4: 0.4 to 3.5 m above the substratum, depending on the tidally variable water depth). Stations 3 and 4 were sampled only when sperm were expected to be present.

At each site, sets of three replicate egg baskets (spaced ~0.1 m apart vertically) were deployed at each station and depth and retrieved 24 h (1999 only), 48 h, or (on only three occasions) 72 h later. In 1999, baskets contained 500 µl of eggs (~7600 eggs) from one female, and in 2000 they contained 800 µl of eggs (mean number ± SE: 11216 ± 787 eggs) pooled from two to three females. Laboratory controls (200 µl of eggs in 10 ml aged seawater) were used to determine the incidence of fertilization membranes prior to basket deployment (presumably reflecting sperm contamination) and at the time of retrieval (presumably reflecting false membranes). To determine fertilization levels, 300 eggs per basket or vial were randomly subsampled and scored in three categories: unfertilized, presence of a fertil-

ization envelope, or development through a later stage (2–64 cells, unhatched/hatched blastula, gastrula). Eggs with fertilization envelopes present were judged to have been fertilized only if the sample also contained later developmental stages. From 41% of baskets (181 out of 441), fewer than 300 eggs were retrieved; in these cases, all retrieved eggs were scored. For the calculation of mean fertilization levels, only baskets with more than 50 retrieved eggs were used, resulting in a loss of replicates at some sites and times.

We estimated the approximate distribution of fertilization events during a sample interval from the distribution of developmental stages in a sample and the known rate of development to each stage. We used Stephens' (1972) developmental times for *S. droebachiensis* at 4°C from fertilization to 32-cell stage (2-cell: 5 h; 4-cell: 8 h; 8-cell: 10.5 h; 16-cell: 14 h; 32-cell: 18 h). From the 64-cell stage to gastrulation, we used our own observations of developmental times (64-cell: 21 h; blastula: 24 h; hatching: 40 h; early gastrula: 48 h). We calculated the distribution of fertilizations (%) in time as the percent at each stage (*i.e.*, of a certain age, in h) of all embryos detected (pooled from three replicate baskets).

To establish the extent to which spawning had occurred during the 2000 sampling period, we collected sea urchins for analysis of gonad index (wet weight of gonads as a percentage of total wet body weight) from ChC ($n = 10$) and CIC ($n = 11$) on April 7 and 11, 2000, respectively.

Results

Egg longevity

Egg viability in aged seawater in the laboratory (as assayed by fertilization with fresh sperm) varied significantly among time intervals and females in both experiments (Fig. 1). In experiment 1 (February 28 to March 2, 2000), the effects of both Female ($F_{3,36} = 5.68, P = 0.003$) and Time ($F_{2,36} = 8.94, P < 0.001$) were significant, but the interaction between the two main factors was not ($F_{6,36} = 1.74, P = 0.14$). Post-hoc comparisons revealed that fertilization levels were significantly lower for female 2, but similar for females 1, 3, and 4 (SNK-test, $P < 0.05$; Fig. 1A). Fertilization levels were highest at 0 h, similar at 24 and 48 h (SNK, $P > 0.05$), and significantly lower by 72 h (SNK, $P < 0.05$). In experiment 2 (March 28 to April 1, 2000), there were again significant Female ($F_{3,60} = 18.0, P < 0.001$) and Time ($F_{4,60} = 273, P < 0.001$) effects, as well as a significant interaction between the two main factors ($F_{12,60} = 32.9, P < 0.001$). Fertilization of eggs from females 1 and 4 remained relatively high at 72 h, while levels declined markedly for females 2 and 3 (Fig. 1B). For females 1 and 2, fertilizations dropped to very low levels by 96 h, while fertilizations for females 3 and 4 were higher at 96 h than at 72 h (Fig. 1B). Of a total of 36 control samples

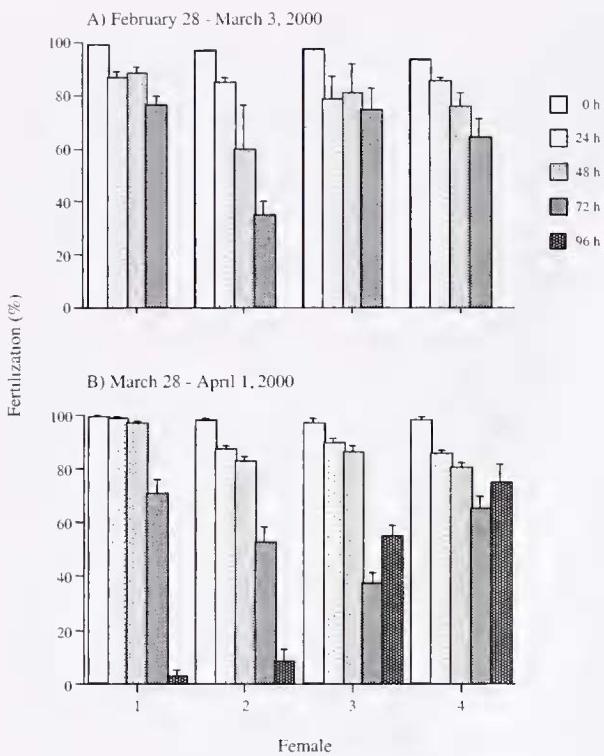


Figure 1. Mean (+SE) fertilization levels (%) over time of eggs from four female sea urchins (A) at the beginning (experiment 1) and (B) in the middle (experiment 2) of the spawning season. Replication is four vials for each female/time combination (except experiment 1 at 0 h; replication = 1).

(16 and 20 in experiments 1 and 2, respectively), 5 had 0.3% false fertilization envelopes and 1 had 0.7%.

In spite of the significant variation among sample times and females in both experiments, egg viability was basically quite high for 48 to 72 h (Fig. 1). With the exception of female 2 in experiment 1, more than 75% of eggs held in aged seawater in the laboratory were viable for 48 h (Fig. 1). At 72 h, viability was in the 50%–75% range for eggs from 6 of the 8 females (Fig. 1).

Cumulative fertilization level (15 min vs 3 h)

When eggs in baskets were exposed to a continuous sperm supply from a spawning male, fertilization levels increased from 15 min to 3 h at distances of 0.3 and 1.0 m downstream from the male, but remained similar over time at 2.6 m (Fig. 2). In the 15-min samples, fertilization decreased with distance from 0.3 to 1.0 m, but remained similar between 1 and 2.6 m (Fig. 2). In the 3-h samples, fertilization decreased monotonically with distance. The two-way ANOVA indicated significant Time ($F_{1,39} = 31.3$, $P < 0.001$) and Distance ($F_{2,39} = 40.1$, $P < 0.001$) effects, as well as a significant interaction between the two main factors ($F_{2,39} = 4.87$, $P = 0.013$). In 5 out

of 8 trials, the male still had sperm on its test at the end of the 3-h deployment, suggesting that fertilization would have continued well beyond the end of our sample interval.

Upstream controls for ambient sperm levels (Fig. 2) generally had $\leq 0.3\%$ fertilization except in trials 1, 6, and 7 when fertilization levels reached 5.3%, 9.0%, and 2.0%, respectively. We attribute fertilizations in trial 6 to a large boat wake that probably created oscillatory water motion and transported sperm towards the upstream control sample immediately before retrieval of the 15-min samples, and we attribute fertilizations in trial 7 to false envelopes (see below). Fertilizations in trial 1 could not be attributed to any obvious cause, and the recorded value was subtracted from the fertilization levels recorded in experimental baskets for that trial.

The apparent absence of a decline in fertilization between the 1- and 2.6-m samples at 15 min and the lack of an increase in fertilization between the 15-min and 3-h samples at 2.6 m are both attributable to one exceptional sample. During trial 5, we recorded a fertilization level of 48% at 2.6 m at 15 min, while values in other trials ranged only from 0.0% to 3.3% (mean \pm SE %: $1.4\% \pm 0.5\%$; $n = 6$) at 15 min and from 3.7% to 15.3% ($6.9\% \pm 1.8\%$; $n = 6$) at 3 h. If this outlier is excluded, fertilization declines from 1 to 2.6 m at 15 min and increases from 15 min to 3 h at 2.6 m.

In laboratory controls, fertilization levels were always very high at the beginning (mean \pm SE: $94.6\% \pm 1.7\%$; $n = 8$) and the end ($94.8\% \pm 1.6\%$; $n = 8$) of a trial. Controls for sperm contamination or false fertilization envelopes mostly indicated 0% envelopes (15 min, $0.3\% \pm 0.2\%$; 3 h, $0.5\% \pm 0.4\%$; $n = 8$) except in trial 7 where

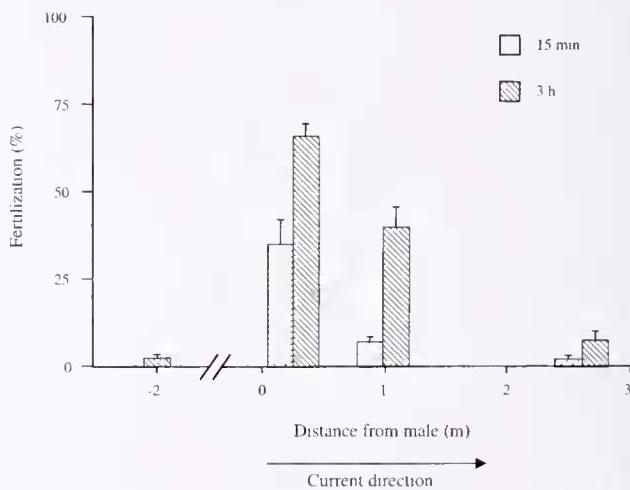


Figure 2. Fertilization as a function of distance and duration of sperm exposure in the field experiment. Mean (+SE) fertilization levels (%) are reported for each time/distance combination. Spawning male is located at 0.0 m mark. Upstream basket was retrieved after 3 h (hatched bar); downstream baskets after 15 min (stippled bars) or 3 h. Replication is 8 trials, except 7 trials for 2.6 m after 15 min, and 6 trials for 2.6 m after 3 h.

1.7% and 3.3% envelopes were found after 15 min and 3 h, respectively. These percentages were subtracted from the fertilization levels recorded in the field for that trial.

Current velocities varied widely during trials 2 through 7 and ranged mainly from 0.08 to 0.20 $\text{m} \cdot \text{s}^{-1}$ (Fig. 3). Mean velocities varied 5-fold among trials during the initial 15-min period (from 0.026 to 0.130 $\text{m} \cdot \text{s}^{-1}$) but were quite similar over 3 h (from 0.121 to 0.155 $\text{m} \cdot \text{s}^{-1}$).

Sperm availability in nature

In both years of the survey (1999, 2000) and at both sites (ChC, CIC), no fertilizations were recorded during most of the sample intervals. However, in both years several sperm-release events of variable magnitude were detected. In 1999 at ChC (only station 3 surface was sampled), fertilizations occurred on March 5 (mean time-integrated fertilization level 4.7%), March 23 (57.3%), March 31 (6.6%), and April 1 (24.6%). In 2000 at ChC, fertilizations occurred on February 19 (station 1 only, 39.5%), March 10 (station 1, 10.3%; station 2, 9.3% surface; no bottom samples were deployed and no fertilization was detected at station 3), March 19 (station 1, 62.3%; station 2, 34.3% surface and 11.3% bottom; station 3, 30.4% surface and 5.3% bottom), and March 29 (station 1, 3.4%; station 2, 4.6% surface; station 3, 4.5% surface; no bottom samples were deployed). At CIC (sampled only in 2000), fertilizations were detected on March 10 (station 1, 24.1%; no fertilization was detected

at station 2; stations 3 and 4 were not sampled), March 17 (station 1, 27.7%; station 2, 10.4%; station 3, 26.2%; station 4, 3.7%), and April 3 (station 1, 6.9%; station 2, 3.3%; stations 3 and 4 were not sampled).

In laboratory controls, fertilization levels were always very high at the start of each sample interval (mean \pm SE %: 1999, 96.7% \pm 0.7%, $n = 19$; 2000, 93.8% \pm 0.9%, $n = 20$). Controls for false fertilization envelopes (stored in the laboratory and fixed upon retrieval of the corresponding field sample) had very low levels of false envelopes (1999, 0.8% \pm 0.5%, $n = 16$; 2000, 0.2% \pm 0.1%; $n = 20$).

Based on the distribution of developmental stages (two-cell to early gastrula) at the time of collection, we estimated that the temporal fertilization pattern varied markedly among the major sperm release events that we detected (Figs. 4–6). Because the discrete developmental stages that we scored are separated by longer time intervals later in development, the 24-h sample interval utilized in 1999 at ChC produced far better resolution of the time of fertilization (~ 3 h) than did the 48- to 72-h intervals employed in 2000 (~ 3 -h resolution for the 24 h immediately preceding sample collection, but ~ 10 h for the portion of the interval >24 h prior to collection). In 1999, fertilizations occurred in fairly continuous trickles over about 48 h (March 3–5; Fig. 4A) or 24 h (March 22–24; Fig. 4B) or in two distinct pulses of similar magnitude about 24 h apart (March 30–April 1;

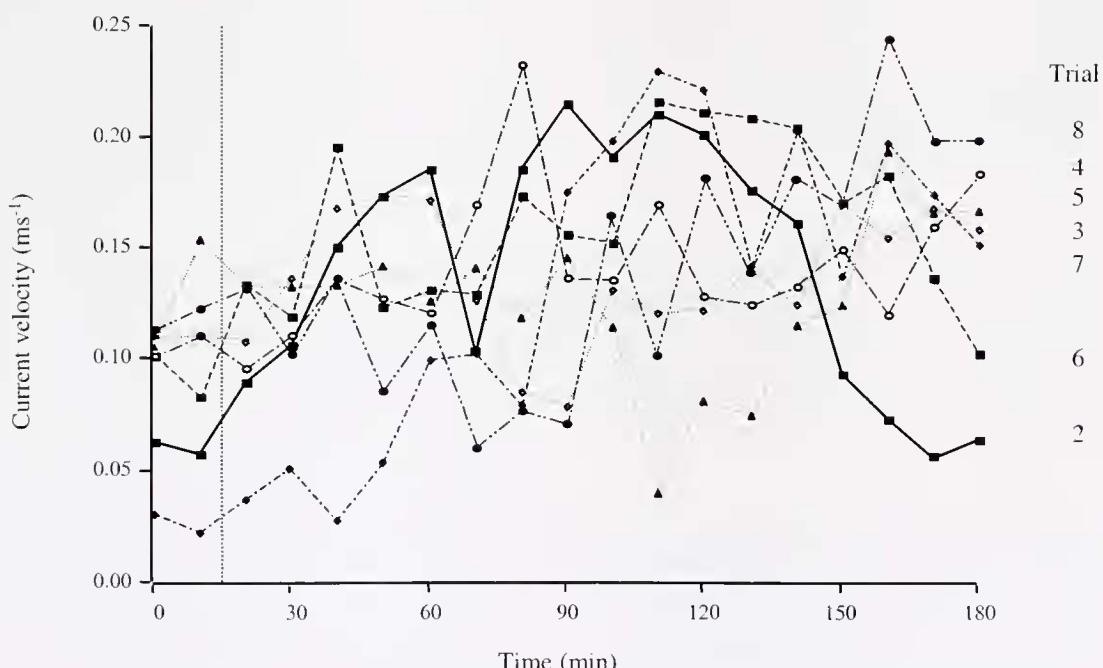


Figure 3. Current velocity ($\text{m} \cdot \text{s}^{-1}$) during seven trials of the field fertilization experiment. Vertical dashed line indicates 15-min interval. Trial 2: each point is one measurement; trials 3–8: each point is mean of 8 measurements collected as two sets of 4 measurements at 15-s intervals 5 min apart. Standard errors are omitted for clarity.

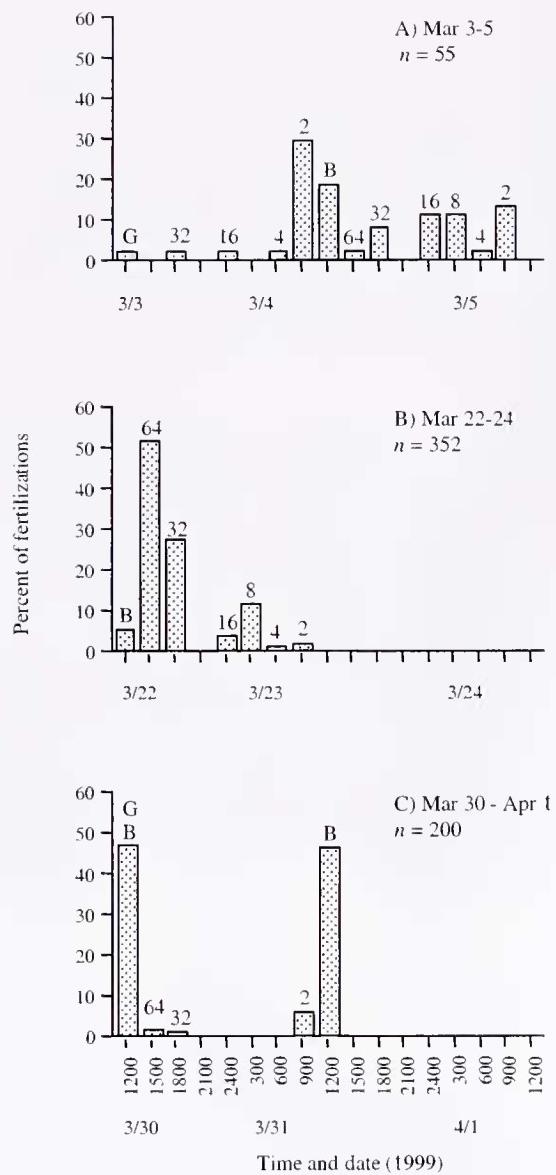


Figure 4. Temporal distribution of fertilizations at Christmas Cove station 3 (surface) during 1999 sample intervals. (A) March 3–5. (B) March 22–24. (C) March 30–April 1. *n*, total number of embryos counted per station (pooled from two to three baskets). Developmental stage corresponding to each inferred fertilization time is indicated above the bar (2, 4, 8, 16, 32, and 64, number of cells; B, unhatched blastula; G, gastrula). When sperm availability extended through two sample intervals (A, C), fertilization times are inferred from both samples.

Fig. 4C). During the most widespread sperm release event in 2000 (March 17–19), fertilizations at 4 of the 5 ChC station/depth combinations were fairly evenly spread over about 24 h (Fig. 5A, B, D, E), but at the fifth station/depth combination virtually all sperm arrived during a much shorter time interval (Fig. 5C). Although the distribution of fertilizations in three apparent pulses at the four stations and depths (Fig. 5A, B, D, E) is an artifact of lower temporal resolution later in development, resolution was nevertheless

sufficient to distinguish fairly consistent sperm availability from a single, shorter pulse (Fig. 5C). During the corresponding fertilization event at CIC (March 15–17, 2000), fertilizations at stations 1 to 3 were also distributed over about 24 h (Fig. 6B–D). At CIC, fertilizations in the March 8–10, 2000 event at station 1 occurred in two major and one minor pulse spread over about 27 h (Fig. 6A).

Sea urchins collected at the end of the field survey at ChC and CIC had intermediate to high gonad indices relative to levels previously recorded for *S. droebachiensis* off the Maine coast (Cocanour and Allen, 1967). Mean gonad indices (\pm SE) were as follows: ChC females, 19.9% \pm 4.7% (*n* = 3), males, 12.3% \pm 2.5% (*n* = 7); CIC females, 14.2% \pm 8.8% (*n* = 5), males, 9.1% \pm 7.0% (*n* = 6). Consequently, additional spawning is likely to have taken place later in the season, after sampling ceased.

Discussion

More than 75% of eggs of the temperate sea urchin *Strongylocentrotus droebachiensis* were generally viable for 48 h when kept in the laboratory in aged (but otherwise untreated) seawater, and viability through 72 h ranged from 50% to 75% in most females (Fig. 1). Subsamples from later time intervals that were isolated prior to formaldehyde addition continued to develop normally through gastrulation (unpub. data). Consequently, fertilization appears to be a reasonable assay of true egg longevity, and does not merely indicate a prolonged ability to elevate a fertilization envelope. Overall, our egg longevity values are greater than earlier estimates of 8 h in sterilized seawater (Wahle and Peckham, 1999) and 24 h in filtered seawater (Pennington, 1985), but shorter than the 1–2 weeks for sea urchin eggs kept under axenic conditions (Epel *et al.*, 1998). Variation both within and among studies, coupled with observations of egg damage in our field surveys, suggests that egg viability is not static but is instead affected by a combination of endogenous and exogenous factors. Variation in egg longevity among the different females in our laboratory experiment (Fig. 1) illustrates the presence of endogenous individual variation. Epel *et al.* (1998) attribute the extreme egg longevity in their study to the removal of bacterial contaminants that can cause the lysis of eggs under laboratory conditions. We observed another form of exogenous damage to eggs in some field samples subject to rough weather (pers. obs.), especially when sediment particles became trapped in the egg baskets. Although damage from sediment abrasion may simply represent a basket artifact, it may also be indicative of a type of egg damage that occurs in nature. Factors controlling egg longevity may ultimately prove to play a significant role in determining fertilization levels in natural spawns.

When we exposed eggs to sperm slowly diffusing from a spawning male's spines and tests (which functionally pro-

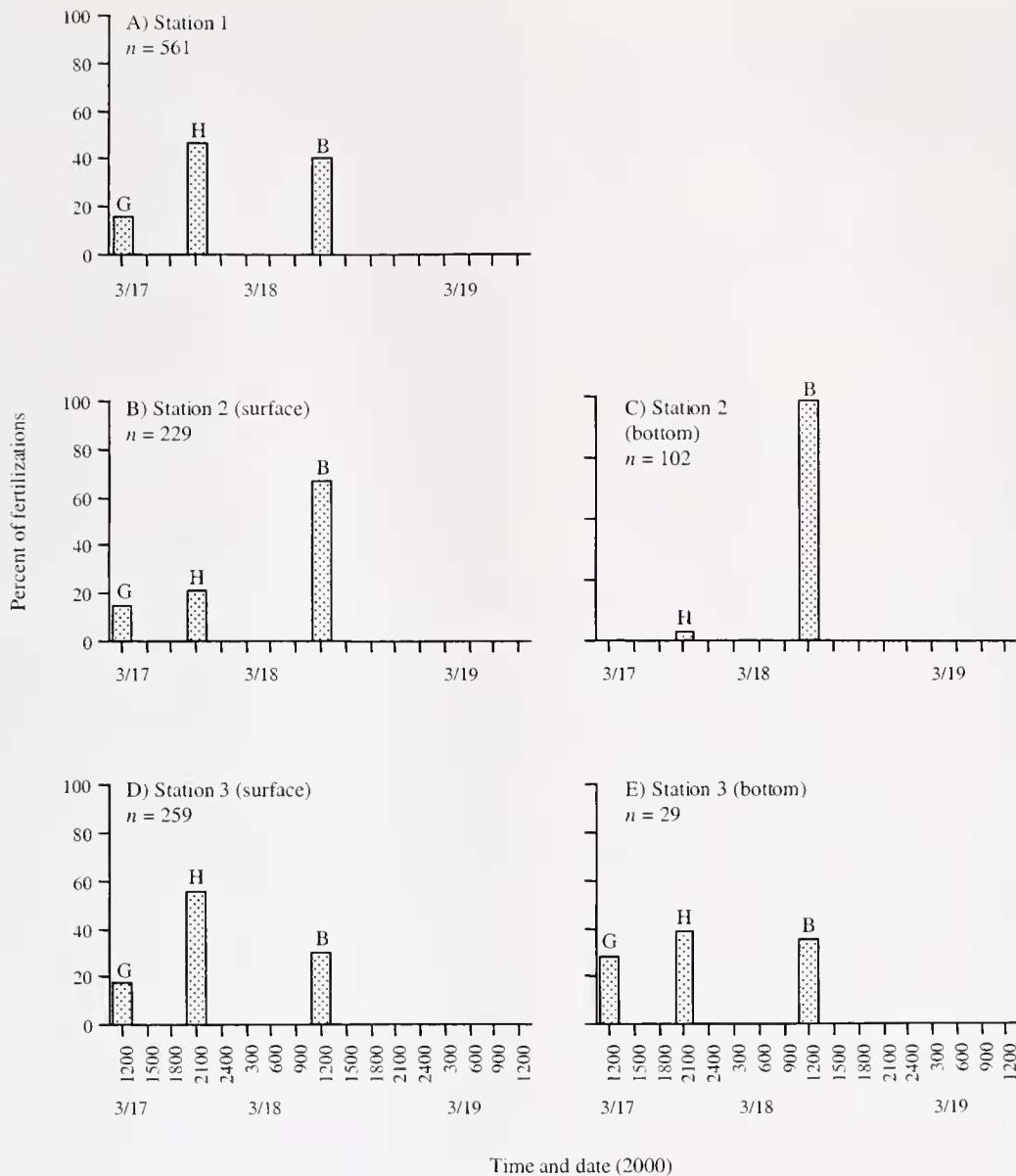


Figure 5. Temporal distribution of fertilizations at Christmas Cove during sample interval March 17–19, 2000. (A) Station 1. (B) Station 2 (surface). (C) Station 2 (bottom). (D) Station 3 (surface). (E) Station 3 (bottom). *n*, total number of embryos counted per station (pooled from three baskets). Developmental stage corresponding to each inferred fertilization time is indicated above the bar (B, unhatched blastula; H, hatched blastula; G, gastrula).

longs male spawning duration, even though sperm release *per se* may have been short in duration), we recorded higher fertilization levels with time at most downstream locations (Fig. 2). Even though current velocities at our experimental site were often considerable and always exceeded the $0.13 \text{ m} \cdot \text{s}^{-1}$ sperm diffusion threshold suggested by Thomas (1994) during at least some portion of each trial (Fig. 3), 62% of our males still had substantial sperm clinging to their spines and tests when retrieved at the end of the 3-h period (similar observations are reported in Pearse *et al.*,

1988, for a female *S. droebachiensis*). Hence even our 3-h experiment probably underestimates the total time-integrated fertilization levels of fixed-position eggs downstream of a spawning male. Short-term fertilization experiments that use sperm-filled syringes to mimic males (Pennington, 1985; Levitan, 1991; Levitan *et al.*, 1992; Wahle and Peckham, 1999) completely bypass this effect.

The relatively long period of egg viability in *S. droebachiensis* makes it feasible to use sperm-permeable baskets of eggs to assess spatial and temporal patterns of sperm

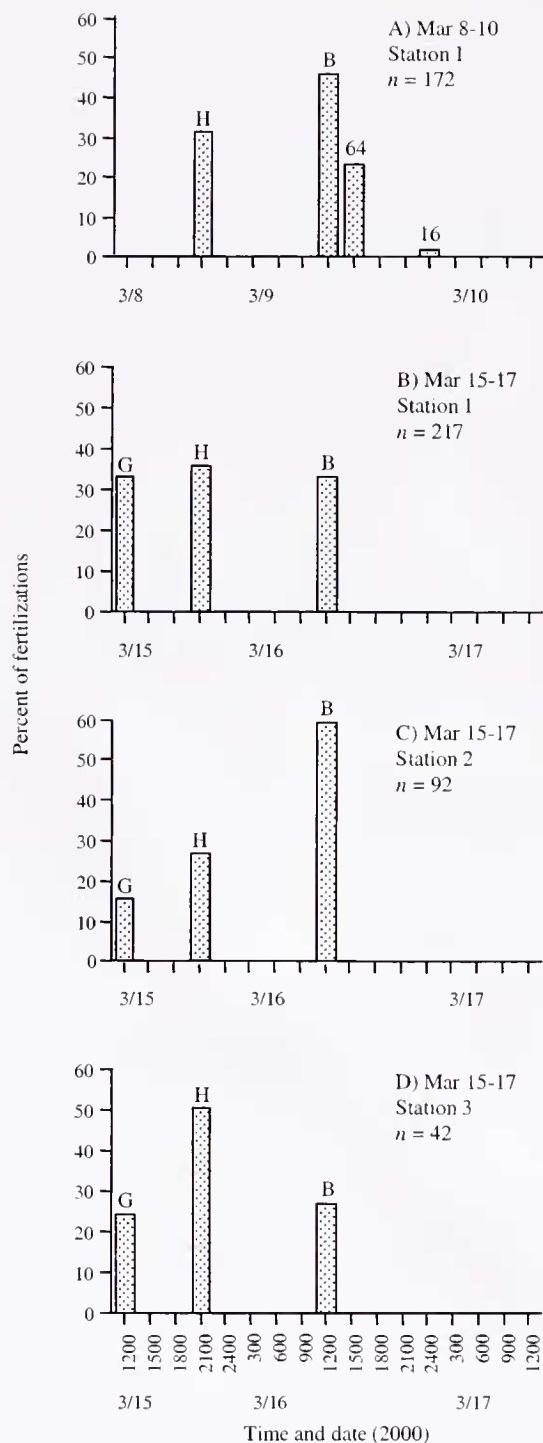


Figure 6. Temporal distribution of fertilizations at Clarks Cove during sample intervals March 8–10 (A) and March 15–17, 2000 (B–D). (A) Station 1. (B) Station 1. (C) Station 2. (D) Station 3. *n*, total number of embryos counted per station (pooled from three baskets). Developmental stage corresponding to each inferred fertilization time is indicated above the bar (32 and 64, number of cells; B, unhatched blastula; H, hatched blastula; G, gastrula).

availability in nature. Our preliminary application of this method detected several sperm-release events in two small populations, one occurring naturally (ChC) and one estab-

lished experimentally (CIC). Several features of the detected sperm-release events are noteworthy. First, total time-integrated fertilization levels were highly variable, ranging from 3.3% to 62% fertilization (when sperm were detected). We emphasize that our experimental design is a hybrid between an experiment and a true survey, because egg position was under experimental control but sperm release occurred naturally. Furthermore, we do not know the actual location of the male or males that spawned, though repeated observations of the distribution of sea urchins during the survey suggest that spawners were likely to be near stations 1 or 2 at both sites (animals were never present at station 3 at ChC, or stations 3 or 4 at CIC). Given these considerations, great care should be exercised when interpreting the absolute fertilization levels reported here. Variation in fertilization levels among sample dates probably reflects the number and proximity of spawning males, but it may be erroneous to conclude that either the higher or lower levels assayed truly represent fertilization levels in natural spawns.

Second, the spatial sampling scheme adopted during 2000 permits some inferences about the spatial scale of sperm availability. Some sperm-release events appear to be highly localized (*e.g.*, ChC, February 19, 2000, station 1 only; CIC, March 10, 2000, station 1 only), with eggs at one station fertilized while eggs a few meters away were not. These sperm distributions are consistent with a pattern of localized sperm availability as indicated by field fertilization experiments conducted with sea urchins (Pennington, 1985; Levitan, 1991; Levitan *et al.*, 1992; Wahle and Peckham, 1999). At other times sperm were present throughout much larger areas (*e.g.*, March 19, 2000, at ChC, March 17, 2000, at CIC). During these widespread sperm-availability events, fertilization levels were often appreciable in much of the site (along a 12-m linear transect at ChC, and within an ~72-m² triangle at CIC). Regardless of the actual location or number of males spawning, the spatial distribution of fertilizations is more extensive than predicted by simple field fertilization experiments (though more consistent with predictions from a whole-population spawning model; Levitan and Young, 1995).

Third, the single sample that provides fertilization levels at different depths at multiple stations (ChC stations 2 and 3, March 19, 2000) indicated higher fertilization levels near the surface than near the bottom. At least close to shore in shallow water, spawned sperm may tend to be concentrated near the surface rather than near the bottom. The distribution is particularly interesting because eggs are negatively buoyant and hence generally assumed to remain near the bottom.

The distribution of developmental stages in retrieved samples during our 1999 field survey indicated that fertilizations during natural sperm release events may accrue over as much as 48 h (Fig. 4). The decreased temporal resolution

in our 2000 survey nevertheless produced temporal patterns that were consistent with fertilization over about 24 h in most samples (Figs. 5, 6). Fertilizations that occur over extended time periods could be the result of continuous extrusion of sperm from a single male's gonopores, the diffusion of sperm from a gamete reservoir that has accumulated on a male's test, or spawning by multiple males at different times.

Our approach of using egg baskets in field experiments and surveys can potentially be criticized because eggs were held stationary at relatively high concentrations instead of being allowed to move and disperse with the currents. If eggs are rapidly transported away from the female during natural spawns, and thus quickly diluted, egg longevity would be less important in determining cumulative fertilization levels than suggested by our results. But because eggs are spawned in a viscous mass that tends to remain on the test (Thomas, 1994), restraining eggs in baskets may adequately approximate natural spawns and provide a good estimator of fertilization levels under a range of flow conditions. Future work should address the outstanding question of where eggs are actually fertilized: in the egg mass on a female's test (*i.e.*, a fixed location), as they transition from that mass into the water column (still essentially a fixed location), or in the mainstream of flow. The answer to this question is likely to vary with habitat and flow regime.

Our study suggests that details of the reproductive biology of sea urchins can potentially have considerable effects on fertilization levels in the field and that caution should be used when extrapolating fertilization levels in natural spawns from experiments that circumvent these apparent adaptations. We suggest that successful fertilization in sea urchins may result not only from short-term exposure to highly concentrated sperm from a nearby male, but also from long-term exposure to more dilute sperm from a number of more distant males. This proposed scenario is similar to our understanding of fertilization in brooding invertebrates with mechanisms to capture dilute sperm (*e.g.*, Yund, 1998; Bishop, 1998), and has also been suggested for tube-dwelling broadcasters that can move sperm-containing water past spawned eggs (M. E. Williams and M. G. Bentley, University of St. Andrews, Scotland, unpub. obs.). We suggest that fertilization in other broadcast-spawning invertebrates may not be fundamentally all that different.

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